2594-Pos Board B580

Influence of Quantum Dot Labels on Single Molecule Movement in the Plasma Membrane

Mathias P. Clausen, Christoffer Lagerholm.

Single particle tracking results are very dependent on the probe that is used. In this study we have investigated the influence that functionalized quantum dots (QDs) have on the recorded movement in single molecule tracking experiments of plasma membrane species in live cells. Potential issues in labeling single molecules with QDs (and other particles e.g. gold particles) are induction of cross-linking of the target molecules, which can cause activation of signaling pathways or reduced mobility, and steric hindrance as a result of the probe size. Cross-linking can be a result of the multivalent functionalization tag (e.g. streptavidin (sAv)) or the presence of multiple mono- or multivalent functionalization tags per QD. In this work, we have compared commercially available sAv-QDs of different sizes with custom prepared Co enzyme A (CoA)-QDs both targeting a GPI-anchored protein modified with either a biotin ligase acceptor peptide (BLAP) or an acyl carrier protein (ACP) tag, respectively. Trajectories of the differently labeled GPI-anchored molecules were recorded simultaneously in dual-color experiments at rates of ~25 -~1500 Hz. Knowing the effect of different labels is of utmost importance for simultaneous investigations of different plasma membrane species in order to discriminate the effect of the label from differences in movement of the target molecules.

Molecular Mechanics & Force Spectroscopy

2595-Pos Board B581

Reconstruction of Energy Profiles from Rupture Times: Application to Force Spectroscopy Experiments

Pak-Wing Fok, Tom Chou.

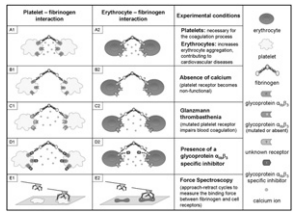
We explore the mathematical and numerical aspects of reconstructing a potential energy profile of a molecular bond from its rupture time distribution. While reliable reconstruction of gross attributes, such as the height and the width of an energy barrier, can be easily extracted from a single first passage time (FPT) distribution, the reconstruction of finer structure is ill-conditioned. More careful analysis shows the existence of optimal bond potential amplitudes (represented by an effective Peclet number) and initial bond configurations that yield the most efficient numerical reconstruction of simple potentials. Furthermore, we show that reconstruction of more complex potentials containing multiple minima can be achieved by simultaneously using two or more measured FPT distributions, obtained under different physical conditions. For example, by changing the effective potential energy surface by known amounts, additional measured FPT distributions improve the reconstruction. We demonstrate the possibility of reconstructing potentials with multiple minima, motivate heuristic rules-of-thumb for optimizing the reconstruction, and discuss further applications and extensions.

2596-Pos Board B582

Identification of the Fibrinogen Receptor on Human Erythrocyte by AFM-Based Force Spectroscopy

Filomena A. Carvalho, Simon Connell, Gabriel Miltenberger-Miltenyi, Sónia V. Pereira, Alice Tavares, Robert A.S. Ariëns, **Nuno C. Santos**.

High levels of fibrinogen in circulation are associated with increased erythrocyte aggregation and higher incidence of cardiovascular pathologies. Platelets are known to have a fibrinogen integrin receptor (the membrane glycoprotein complex $\alpha_{IIb}\beta_3$). We demonstrate, by force spectroscopy measurements using an atomic force microscope (AFM), the existence of a single molecule interaction between fibrinogen and an unknown receptor on the erythrocyte membrane, with a lower but comparable affinity relative to platelet binding. Its inhibition by calcium depletion and by eptifibatide (an $\alpha_{IIb}\beta_3$ specific inhibitor) indicates that it is an $\alpha_{IIb}\beta_3$-related integrin. Results obtained for a Glanzmann thrombastenia (a rare hereditary bleeding disease caused by $\alpha_{IIb}\beta_3$ deficiency) patient show an impaired fibrinogen-erythrocyte binding. Correlation with genetic sequencing data demonstrates that one of the units of the fibrinogen receptor on erythrocytes is a product of the expression of the β_3 gene, found to be mutated in this patient. This work demonstrates and validates the applicability of AFM-based force spectroscopy as a highly sensitive, rapid and low operation cost nanotool for the diagnostic of hematological diseases, with an unbiased functional evaluation of their severity.



[Carvalho *et al.* (2010) *ACS Nano*, **4**:4609-20]

2597-Pos Board B583

Nanomechanical Properties of Supported Lipid Bialyers

Shan Zou.

Lipid reorganization induced morphology alteration in asymmetric bilayers prepared by Langmuir-Blodgett (LB)/ Langmuir-Schaeffer (LS) method was directly visualized by means of the atomic force microscopy (AFM) and fluorescence imaging. The LB monolayer in the bilayer system was prepared using binary mixture of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dipalmitoyl -sn-glycero-3-phosphocholine (DPPC) in 1:1 molar ratio, with 0.2 mol% Texas Red 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (TR-DHPE dye). The shapes of the flower like DPPC-enriched domains were found to be dependent on the preparing temperatures. These domains were well maintained after the LS layer of DOPC was deposited. The contrast of these domains has changed from the original dark to bright, and turned dark again after the sample stayed in water for several hours. Along with the contrast reverse, a third phase was observed to separate out within the domains, exhibiting a much taller height than the outside-domain areas. Mechanical properties of these regions were detected by means of force spectroscopy. The increased heights within DPPC domains and the rapid contrast reverse suggest that not only DOPC but also DPPC lipids have reorganized in the LB/LS bilayer, stimulated the morphology changes.

2598-Pos Board B584

Investigation of Intercellular Adhesion of Red Blood Cells by Means of Holographic Optical Tweezers and Single Cell Force Spectroscopy

Patrick Steffen, Achim Jung, Duc Bach Nguyen, Ingolf Bernhardt, Torsten Mueller, Lars Kaestner, Christian Wagner.

Red blood cells are a major component of blood clots which form physiologically as a response to injury or pathologically in thrombosis. The active participation of RBCs in thrombus solidification was previously proposed, but not yet experimentally proven. Holographic optical tweezers and single cell force spectroscopy were used to study potential cell-cell adhesion in between RBCs. Irreversible intercellular adhesion of RBCs could be induced by stimulation with lysophosphatidic acid (LPA), a compound known to be released by activated platelets. We identified Ca^{2+} as an essential player in the signaling cascade by directly inducing Ca^{2+} influx using A23187. The elevation of internal Ca^{2+} concentration leads to intercellular adhesion of RBCs similar to that induced by LPA stimulation. Based on single cell force spectroscopy adhesion of the RBCs was identified to be approximately 100 pN, a value large enough to be of significance inside a blood clot or in pathological situations like the vasco-occlusive crisis in sickle cell disease patients.

2599-Pos Board B585

Mechanical Stabilization of Membrane Proteins using Compatible Solutes

Arpita Roychoudhury, Roland Reinehr, Dieter Häussinger, Filipp Oesterhelt.

Mechanical single molecule techniques offer exciting possibilities for investigating protein folding and stability in native environments at sub-nanometer resolutions. The single molecules without inherent symmetry can directly be monitored in their physiological conditions using atomic force microscopy (AFM). Recent developments in AFM enable us to go beyond the ensemble average and measure the behavior of individual molecules.

In nature, compatible solutes (organic osmolytes) are used for protecting cells against high osmotic stress. They are compatible with cell metabolism even at molar concentrations. The influence of ectoine (1M), betaine (1M) and taurine (0.4M) on the mechanical properties of bacteriorhodopsin (BR) has been investigated by single molecule force spectroscopy. Unfolding experiments taking BR as a model system revealed that these compatible osmolytes increase the tendency of the polypeptide to coil, thereby decreasing its persistence length. This allows us to explain the mechanism of interaction between the unfolded polypeptide chain and the osmolyte. The osmolytes are expelled from the protein surface due to the increase in chemical potential of the stretched (denatured) state collapsing the protein into a more compact structure.

These information and approaches provide basis for our further studies regarding the effects of compatible solutes on other membrane proteins of medical importance (particularly in case of liver diseases) which can directly resolve transient intermediate states and multiple reaction pathways, and thus are uniquely powerful in characterizing the complex dynamics of protein folding. Thus, this study is set to provide exciting possibilities in the field of drug development for liver diseases including in vitro rescue of the misfolded proteins and to directly analyze and correlate their structural and functional properties at the sub-molecular level.